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STARCH PROPERTIES OF VARIOUS ZP MAIZE GENOTYPES

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The objective of this study was to investigate molecular and functional properties of starches isolated from ZP maize genotypes of different genetic background. The protein, fat, ash and resistant starch contents were very low. The amylose content in the isolated starches of 10 ZP maize genotypes was characteristic for both types of maize starches, normal and waxy. The waxy type had the highest average molecular weight of amylopectin (4.84 x 10^8 Da). The onset temperature of gelatinisation values of starches of 10 ZP maize genotypes ranged from 62.1ºC to 65.0ºC. The waxy maize starch displayed a significantly higher enthalpy change for gelatinised starch (ΔH=18.1 J/g) than normal maize starches did (ΔH=13.6-15.6 J/g). Rapid Visco Analyser (RVA) profiles of starches of ZP maize genotypes were typical for both types of maize starches, normal and waxy.

KEY WORDS: starch, maize, molecular characteristics, functional properties

INTRODUCTION

Starch is the second largest biomass produced on earth, next to cellulose. It is the most important plant reserve material accumulating in fruits, seeds, roots and tubers in the form of starch granules that provide 70-80% energy consumed by the world population. Commercial starches, used in the food-processing industry, are most often produced from grain of common, waxy and high-amylose maize, then wheat, and different varieties of rice, as well as, from potato, sweet potato and cassava (tapioca starch). Depending on their botanical origin, starches differ in their chemical structure, size and shape of their granules, and consequently in their functional and sensory properties. Compositions of maize (*Zea mays* L.) starch vary depending on genotypes. Normal maize starch contains about 25-30% amylose and 70-75% amylopectin; waxy starch consists mainly of amylopectin and 0-8% amylose; high-amylose starch consists of 40-85% amylose (1). Many starches, such as high-amylose maize starches (2, 3) and sugary-2 starches (4), also contain intermediate components that are branched molecules with smaller molecular weights and longer branch chains than amylopectin. Normal maize starch also contains minor components including lipids (free fatty acids and triglycerides) and little phosphorlipids.

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Amylose is primarily a linear glucan, consisting of α 1–4 linked D-glucopyranose with a few branches (5). The molecular size of amylose varies from 500 anhydroglucose units (AGU) or 500 degree of polymerisation (DP) of high-amylose maize starch (6) to more than 6000 AGU or DP 6000 of potato starch (7). Amylopectin is a highly branched molecule, consisting of α 1–4 linked D-glucopyranose chains that are connected by α 1–6 branch linkages. Furthermore, amylopectin has a very large molecular weight (7×10^7 to 5.7×10^9 Da) (8). Amylopectin in the granule is present in the semi-crystalline structure, whereas amylose is amorphous.

Starch granules possess different types of crystallinity, displaying A-, B- and C-type X-ray patterns, depending on their amylopectin branch chain-length (9). The type A structure is very common in cereals, the type B in raw potato and banana, while the type C is typical for peas and beans. Normal and waxy maize starch granules display A-type X-ray patterns, while high amylose maize starches display B-type. Starches of different polymorphisms are known to display different enzyme digestibility (4, 10, 11, 12). The A-type polymorphic starch is easily digestible, but the B-type and some C-type starches are very resistant to enzyme hydrolysis. Resistant starch (RS) is the portion of starch that is not digested in small intestine, but is fermented by microflora in the colon (13). Four classes of RS have been proposed on the basis of mechanisms of enzyme indigestibility, including inaccessibility of starch to amylases due to physical entrapment (RS1), inherent granular structure of raw starch (RS2), molecular association of amylose or retrogradetion (RS3) and chemical modification (RS4).

In this study, we investigated the starch composition, amylose content, amylopectin branch chain-length distribution, gelatinisation and pasting properties of starches isolated from ZP maize genotypes of different genetic background. In terms of technological value this information will be useful to recognise significant differences in the properties of isolated starches of the selected ZP maize genotypes.

EXPERIMENTAL

Starches of various ZP maize genotypes (ZP 360, ZP 434, ZP 578, ZP 633, ZP 684, ZP 808, ZP 74b, ZP 611k, ZP 704wx, ZP Rumenka) developed at the Maize Research Institute "Zemun Polje", Belgrade, Serbia were used in this study. The starches were isolated by applying a 100-g laboratory maize wet-milling procedure (14).

The moisture, ash, crude protein and crude fat contents of starch were determined using the oven method (15), AOAC Method (16), microKjeldahl method (16) and Soxhlet method (16), respectively. The amylose content was determined by a rapid colorimetric method (17). RS content was determined according to the enzymatic-gravimetric method (18) and McCleary method (19).

The thermal (gelatinisation) characteristics of the isolated starches were studied by using a differential scanning calorimeter (DSC 2920 modulated, TA Instruments, New Castle, DE) (20). Starch (2 mg, dry matter basis (dmb)) was accurately weighed in an aluminium pan, mixed with 6 mg of deionised water and sealed. The sample was allowed to equilibrate for 2 h and scanned at a rate of 5 ºC/min over a temperature range of 25-120ºC. An empty pan was used as the reference.

The Rapid Visco Analyser (RVA-4, Foss North America, Eden Prairie, MN) was used for studying pasting properties (20). Starch suspension (8%, w/w, dmb), in duplicate for each starch sample, was prepared by weighing starch (2.24 g, dmb) into a RVA canister and making up the total weight to 28 g with deionised water. Starch suspension was equilibrated at 30ºC for 1 min, heated at a rate of 6.0ºC/min up to 95ºC, maintained at 95ºC for 5.5 min, and then cooled to 50ºC at a rate of 6.0ºC/min. A paddle rotating speed (160 rpm) was used throughout the entire analysis, except for rapid stirring at 960 rpm for the first 10 s to disperse the starch sample.

The molecular weight distribution of amylopectin was estimated by a high-performance size-exclusion chromatography, HPSEC e.g. HPSEC-MALLS-RI system (coupled with a multi-angle laser-light scattering, MALLS and a refractive index, RI detector). The HPSEC system consisted of a HP 1050 series isocratic pump (Hewlett–Packard, Valley Forge, PA), a multi-angle laser-light scattering detector (Dawn DSP-F, Wyatt Tech. Co., Santa Barbara, CA) and a HP 1047A refractive index detector (Hewlett–Packard, Valley Forge, PA). Shodex OH pak SB-G guard column and SB-806 and SB-804 analytical columns (Showa Denko K.K., JM Science, Grand Island, NY) were used to separate amylopectin from amylose (8).

Statistical analysis. Results were expressed by means of values ± standard error of three separate determinations. Data reported for the starch composition and gelatinisation and pasting properties was assessed by the analysis of variance (ANOVA) and Duncan's multiple range test was used for any significant differences at the $P<0.05$ level between the means. RVA profiles were presented as means of two separate determinations. All the analyses were conducted using statistical software package STATISTICA 8.1. (StatSoft Inc. USA).

RESULTS AND DISCUSSION

The data for the amylose, protein, fat and ash content and the molecular weight of amylopectin in the isolated starches are given in Table 1. The protein content in the starches ranged from 0.11% to 0.29%, pointing out to high quality ("purity") of obtained starches. The highest protein content was obtained in starch samples isolated from ZP 611k and ZP 633, which can be attributed to the high percentage of hard endosperm in these genotypes and the difficulties in the starch extraction. Starch samples isolated from ZP 360 and ZP 704wx had the lowest values for the protein content, as these genotypes had the high proportion of the soft endosperm fraction (21, 22). Although protein, oil and ash are mostly determined by genetic factors (source of starch), the method of isolation (extraction technique) also has a major impact on the “purity” of starch.

The amylose content in the isolated starches of 10 ZP genotypes was characteristic for normal and waxy maize starches. The highest content of amylose was obtained in the starch isolated from ZP 434 (26.0%). The isolated waxy type starch had the lowest value for the amylose content (1.0%). The results concerning the average molecular weight of amylopectin (Mw) of different ZP maize genotypes (Table 1) show that the waxy type of starch had the highest Mw (4.84×10^8), which is consistent with the research carried out by Sang-Ho and Jane (8) who had found a significant difference between Mw of normal

and waxy starch. This difference can be attributed to the fact that all ADP-glucose is used as a substrate for the biosynthesis of amylopectin in waxy starch, whereas the ADP-glucose substrate is partitioning between amylopectin and amylose for normal starch biosynthesis. The lowest Mw was found in the starches of ZP 74b (1.52×10^8) and ZP 633 (1.66×10^8) genotypes. Significant differences in Mw of various ZP genotypes were found (Table 1). The genotypes ZP 434 and ZP 684 were not significantly different in this parameter as well as the genotypes ZP 360, ZP 578 and ZP Rumenka.

Table 1. Contents of amylose, protein, fat and ash, and Mw of the isolated starches

Starch source	Amylose (%)	Protein (%)	Fat (%)	Ash (%)	Mw x 10^8
ZP 74b	25.0 ± 0.3b	0.20 ± 0.00c	0.30 ± 0.02a	0.10 ± 0.00b	1.52 ± 0.05f
ZP 360	23.8 ± 0.2c	0.11 ± 0.05e	0.22 ± 0.02b	0.09 ± 0.01b	2.27 ± 0.07d
ZP 434	26.0 ± 0.2a	0.26 ± 0.04ab	0.05 ± 0.02e	0.01 ± 0.00d	2.87 ± 0.10b
ZP 578	23.5 ± 0.5cd	0.23 ± 0.02b	0.10 ± 0.01d	0.06 ± 0.03bc	2.27 ± 0.05d
ZP 611k	23.2 ± 0.1d	0.29 ± 0.01a	0.08 ± 0.00de	0.15 ± 0.02a	2.68 ± 0.09c
ZP 633	24.0 ± 0.2c	0.28 ± 0.02a	0.09 ± 0.01d	0.04 ± 0.00c	1.66 ± 0.11f
ZP 684	23.3 ± 0.5cd	0.21 ± 0.05ab	0.07 ± 0.01e	0.10 ± 0.02b	2.97 ± 0.11b
ZP 704wx	1.0 ± 0.1e	0.13 ± 0.02e	0.10 ± 0.03d	0.04 ± 0.00c	4.84 ± 0.15a
ZP 808	24.0 ± 0.2c	0.18 ± 0.02cd	0.15 ± 0.02c	0.09 ± 0.02b	1.91 ± 0.03e
ZP Rumenka	23.5 ± 0.3cd	0.18 ± 0.04cd	0.25 ± 0.02b	0.10 ± 0.01b	2.25 ± 0.02d

* Mw-molecular weight of amylopectin

** Means within a column followed by different letters are significantly different ($P<0.05$)

The RS content in native starch of different ZP maize hybrids (Table 2) was very low and ranged from 0.62 to 1.61% and 0.00 to 0.85% depending on the applied methods. The method developed by McCleary gave lower values for the RS content although this method allows a greater deviation for low RS concentrations in samples (<2%).

Table 2. RS content in the isolated starches

Starch source	RS (%) (*AOAC Official Method 991.43*)	RS (%) (*McCleary Method*, 2002)
ZP 74 b	1.14 ± 0.09	0.55 ± 0.20
ZP 360	1.17 ± 0.04	0.70 ± 0.03
ZP 578	1.26 ± 0.15	0.71 ± 0.10
ZP 611 k	1.50 ± 0.21	0.80 ± 0.01
ZP 704 wx	0.62 ± 0.24	0.00 ± 0.00
ZP 808	1.61 ± 0.11	0.78 ± 0.10
ZP Rumenka	1.41 ± 0.07	0.85 ± 0.05

Gelatinisation parameters of the starch isolated from 10 ZP maize genotypes are shown in Table 3. The onset temperature of gelatinisation values of starches of 10 ZP genotypes ranged from 62.1ºC (ZP 74b) to 65.0ºC (ZP 434). In this study there was no relationship between the amylose content and gelatinisation temperatures of the starches (data are not given). The obtained results of the DSC analysis (Table 3) show that the starch

isolated from ZP 74b had significantly lower gelatinisation temperatures (T_0, T_p, T_c) and enthalpy change compared to other samples of native starch which can be explained by the lowest Mw in this sample. It is believed that the amylopectin chain length affects the crystalline structure of amylopectin, and thus the parameters of gelatinisation (20). With the decreasing amylopectin chain length the crystalline structure of amylopectin changes resulting in a lower gelatinisation temperature. The starch isolated from ZP 434 genotype had significantly higher gelatinisation temperatures. The waxy starch isolated from ZP 704wx had very similar gelatinisation temperatures to the normal starches isolated from ZP 360, ZP 633, ZP 684 and ZP Rumenka. The waxy maize starch displayed a signify-cantly higher enthalpy change for gelatinised starch (ΔH=18.1 J/g) than normal maize starches did (ΔH=13.6-15.6 J/g). The highest value of enthalpy change for gelatinised waxy maize starch (ZP 704wx) can be explained by the lowest amylose content and the highest Mw of the starch.

Table 3. Gelatinisation properties of the starch isolated from various ZP maize genotypes*

Starch sources	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
ZP 74b	62.1 ± 0.2d	66.6 ± 0.1e	70.6 ± 0.5e	13.63 ± 0.17d
ZP 360	63.3 ± 0.0bc	68.3 ± 0.1bc	73.4 ± 0.0b	15.01 ± 0.78bc
ZP 434	65.0 ± 0.5a	69.5 ± 0.2a	74.2 ± 0.4a	15.55 ± 0.16b
ZP 578	62.7 ± 0.2c	67.4 ± 0.1d	72.2 ± 0.1c	15.51 ± 0.39b
ZP 611k	64.5 ± 0.5ab	67.9 ± 0.4c	71.6 ± 0.6d	14.20 ± 0.15c
ZP 633	63.3 ± 0.0bc	67.8 ± 0.0c	71.9 ± 0.3c	15.18 ± 0.58bc
ZP 684	63.2 ± 0.3bc	67.7 ± 0.5cd	71.7 ± 0.6cd	14.53 ± 0.29c
ZP 704wx	63.7 ± 0.1b	68.8 ± 0.1b	74.7 ± 0.0a	18.10 ± 1.21a
ZP 808	62.9 ± 0.3c	67.4 ± 0.1d	71.6 ± 0.2d	14.30 ± 0.06c
ZP Rumenka	63.7 ± 0.1b	68.3 ± 0.2bc	73.1 ± 0.4b	14.84 ± 0.35c

* T_0, T_p, T_c and ΔH are onset, peak and conclusion temperature and enthalpy changes of gelatinisation, respectively.
** Means within a column followed by different letters are significantly different ($P<0.05$)

The RVA is considered to simulate food processing and is used to relate functionality to structural properties (23, 24). RVA profiles of the isolated starches of normal ZP 434, popping ZP 611k and waxy ZP 704wx genotypes are given in Figure 1. The profiles of remaining normal starches from seven ZP genotypes were similar in behaviour to the starch of ZP 434 genotype and therefore they are not presented in this paper.

It is observed that RVA profiles of the starches of ZP genotypes were typical for both types of maize starch, normal and waxy (25, 26). Particularly, normal maize starch isola-ted from the selected ZP genotypes had a temperate peak viscosity (approximately about 2200 cP) and a high tendency of increasing viscosity during paste cooling (“setback”) (approximately about 1300 cP) that is a result of a very high retrogradation rate of a line-ar fraction.

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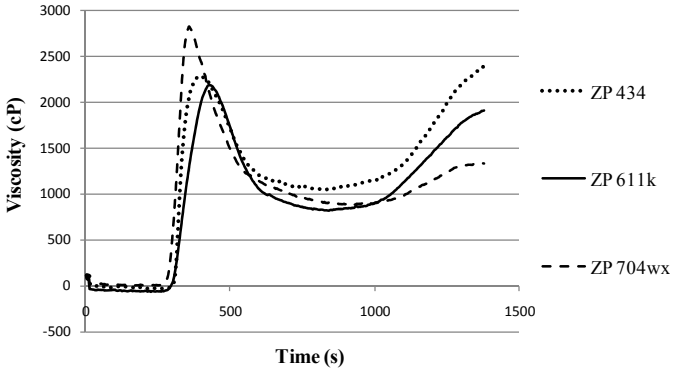

Figure 1. RVA profiles of the selected starches

Starch isolated from ZP 611k genotype (popping maize) had a slightly lower "set-back" viscosity (viscosity at 50°C), which corresponded to a slightly lower amylose content in the sample (23.2%). Waxy maize starch (ZP 704wx) had the peak viscosity of 2822.4 cP and due to a small amount of a linear amylose fraction it had a very low tendency of increasing viscosity during paste cooling (ΔV=429.6 cP).

CONCLUSION

The protein content in the starches was very low (<0.30%), pointing out to high quality ("purity") of obtained starches. The amylose content in the isolated starches of various ZP genotypes was characteristic for normal and waxy maize starches. The waxy type of starch had the highest Mw (4.84 x 10^8 Da). Mw of the normal starch ranged from 1.52 x 10^8 to 2.97 x 10^8 Da. The RS content in native starch of various ZP genotypes was very low (<1.61%). The onset temperature of gelatinisation values of starches of 10 ZP genotypes ranged from 62.1ºC to 65.0ºC. The waxy maize starch displayed a significantly higher enthalpy change for gelatinised starch than normal maize starches did. RVA profiles of the isolated starches were typical for both types of maize starches, normal and waxy. Some starches of specialty hybrids (ZP 74b, ZP 611k and ZP Rumenka) were similar in behaviour to normal starch.

Despite significant differences in the content of amylose, Mw and DSC parameters of some samples there were no significant differences in pasting properties of native starches isolated from the selected ZP maize genotypes with the exception of two specialty genotypes (waxy and popping maize). On the basis of the results it can be concluded the selected dent maize genotypes can be used in wet-milling for production of normal maize starch which may be further modified physically, chemically or enzymatically to meet specific needs and applications.

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ОСОБИНЕ СКРОБА РАЗЛИЧИТИХ ЗП ГЕНОТИПОВА КУКУРУЗА

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Циљ овог рада био је да се испитају молекуларне и функционалне карактеристике скроба изолованих из ЗП генотипова кукуруза различите генетичке основе. Садржај протеина, уља, пепела, као и резистентног скроба био је веома низак. Садржај амилозе у изолованим скробовима био карактеристичан за нормалне односно воштане кукурузне скробове. Воштани тип скроба (ЗП 704wx) имао је највећу просечну молекулску масу амилопектина ($4{,}84 \times 10^8$). Почетна температура желатинизације скроба 10 ЗП генотипова кретала се у распону од 62,1°C до 65,0°C. Воштани кукурузни скроб имао је значајно већу промену енталпије желатинизације (ΔH= 18,1 J/g) у односу на нормалне скробове (ΔH=13,6-15,6 J/g). RVA профили скроба ЗП генотипова били су типични за нормалне, односно за воштани скроб кукуруза.

Кључне речи: скроб, кукуруз, молекуларне карактеристике, функционална својства.

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